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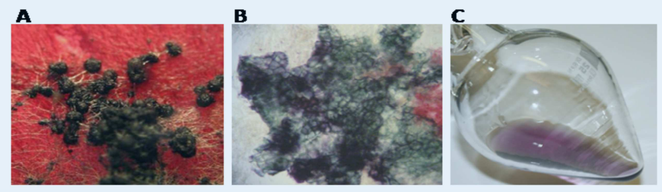

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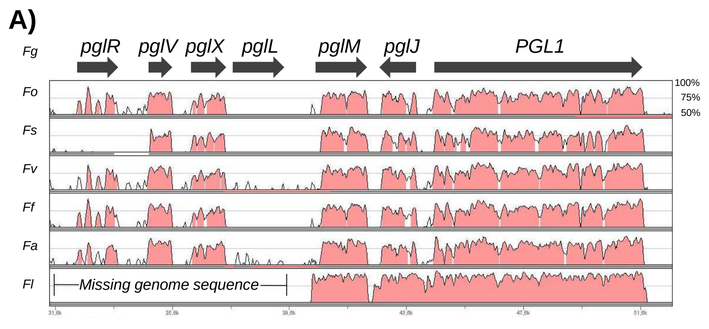
Members of the *Gibberella* genus are characterized by having dark violet perithecia. The chemical nature of the responsible pigments has so far remained unknown. Using targeted over-expression of *PKS3* (*PGL1)* and a cluster specific transcription factor *pglR*, we here identify the pigmetns as bostrycoidines, members of the fusarubin metabolite family.

Identification of a *PGL1* gene cluster

Previous studies [1,2] have show that **PKS3** (*PGL1*) is essential for biosynthesis of the perithecial pigment(s) in *F. graminearum* and *F. verticilioides*.

Using comparative genome analysis (shuffle-LAGAN plots) of seven *Fusarium* species we have identified a conserved PKS gene cluster (Fig. 1A). The cluster encodes classical PKS tailoring enzymes: monooxygenase, O-methyltransferase, dehydrogenases and a Zn_2Cys_6 type transcription factor (***pglR***) (Fig. 1B). The cluster is only expressed in perithecial tissues.



B)

Gene	Predicted function	*Fg*	*Fo*	*Fs*	*Fv*	*Ff*	*Fa*	*Fl*
PGL1	Non-reducing polyketide synthase	√	√	√	√	√	√	√
pglJ	O-methyltransferase	√	√	√	√	√	√	√
pglM	Flavine-dependent monooxygenase	√	√	√	√	√	√	√
pglL	adenylosuccinate lyase (*Ade13 in Sc*)	√	-	-	-	-	-	?
pglX	Zinc-binding dehydrogenase	√	√	√	√	√	√	?
pglV	S-adenosyl-L-homocysteine hydrolase	√	√	√	√	√	√	?
pglR	Zn(II)2Cys2 transcription factor	√	√	-	√	√	√	?

Figure 1: A) Shuffle LAGAN plot (100 bp window) of the PGL1 gene cluster from Fg (*F. graminearum*), Fo (*F. oxysporum*), Fs (*F. solani*), Fv (*F. verticillioides*), Ff (*F. fujikuroi*), Fa (*F. avenacium*) and Fl (*F. langsethiae*). Note that genome assembly in Fl is ongoing. **B)** Enzymes encoded by the PGL1 gene cluster, **C)** Semi-quantitative RT-PCR based analysis of the *pglR* transcription factor over-expression strain**, D)** as C, but for the *PGL1* over-expression strain.

Over-expression of *PGL1* and *pglR*

Based on the small size of perithecia and the classical difficulties of characterizing melanins (heterogenous polymers) we opted to activate the gene cluster in vegetative mycelium by TF and PKS over-expression hoping to identify the chemical nature of the monomers that makes up the perithecial pigment.

Over-expression of *PGL1* and *pglR* (TF) (Fig. 1C&D) resulted in the production and excretion of brown and yellow pigments (Fig. 2A).

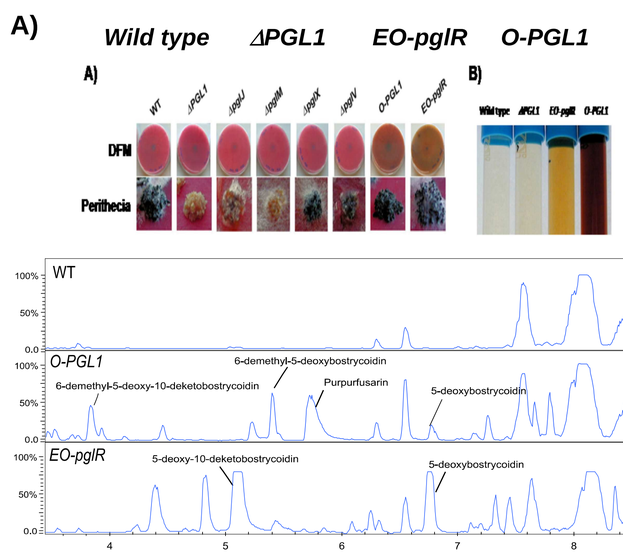


Figure 2: A) Filtrated liquid medium after 10 days of growth at 25ºC with 100 rpm in darkness. The wild type and *ΔPGL1* strains do not excrete any pigments, while the pglR and PGL1 overexpression strain produce. **B)** Analysis by UPLC-HRMS of the metabolites excreted into the growth medium by the wild type (WT), *PGL1* deletion (*ΔPGL1*), *PGL1* over-expression (*O-PGL1*) and *pglR* over-expression (*EO-pglR*) strains.

Chemical analysis, UPLC-HRMS (Figure 2B) and NMR experiments, showed that the *O-PKS3* strain produced **6-O-demethyl-5-deoxy-10-deketobostrycoidin** (1), **6-O-demethyl-5-deoxybostrycoidin** (2) and a purple dimer of 1 (named **purpurfusarin** (3)). While the yellow *EO-pglR* strain accumulated **5-deoxybostrycoidin** (4), **bostrycoidin** (5) and **5-deoxy-10-deketobostrycoidin** (6).

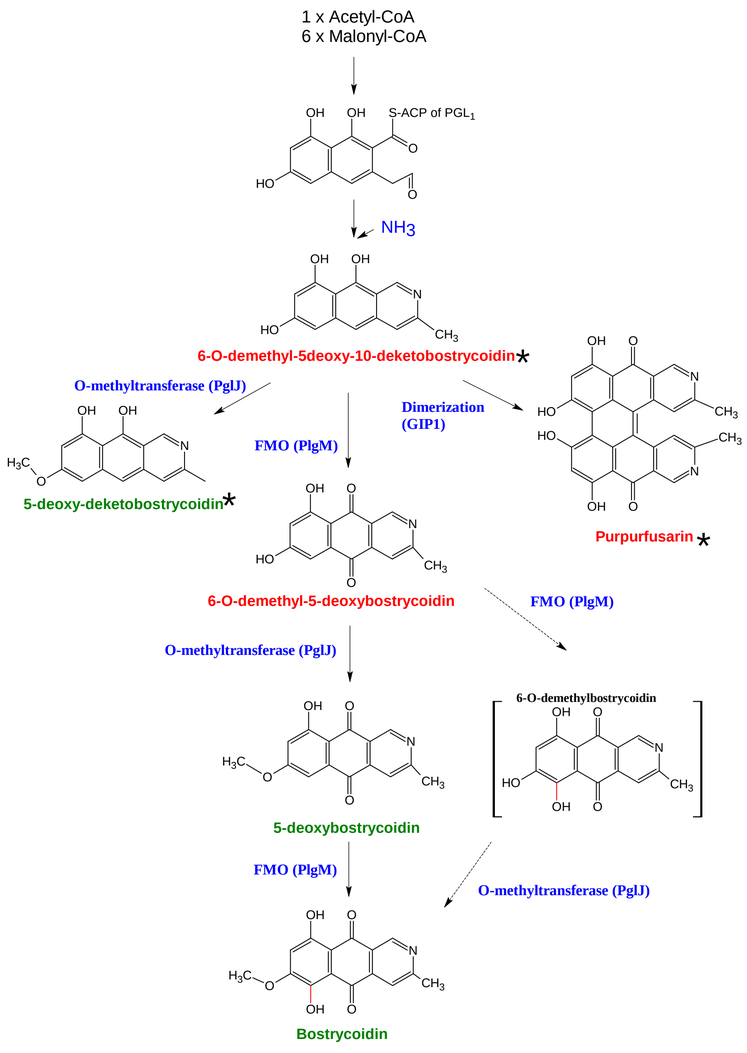


Figure 3: Model for formation of bostrycoidin based on the accumulating metabolites in the *O-PGL1* (shown in red) and the *EO-pglR* (shown in blue) strains. Novel compounds are marked with *.

Biosynthetic model(s)

Based on structure of the accumulating intermediates we have formulated a model for biosynthesis of bostrycoidin in *F. graminearum* (Fig 3). Targeted deletion of *pglJ* and *pglM* has confirmed their involvement in the biosynthetic pathway (data not shown). The origin of the nitrogen atom in the C-ring is currently unknown.

Interestingly the identified monomers all display yellow color at physiological pH while the dimer is pink/violet.

The study shows that when *PGL1* and the corresponding cluster is expressed in vegetative mycelium it results in the production of bostrycoidin pigments. To confirm that the perithecial pigments are bostrycoidins, and rule out an artifact of the overexpression in vegetative mycelium, chemical analysis of perithecia is ongoing.

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References:
[1] Gaffoor I, Brown DW, Plattner R, Proctor RH, Qi W, Trail F. 2005. Eukaryot Cell 4(11):1926-33,
[2] Proctor RH, Butchko RA, Brown DW, Moretti A. 2007, Food Addit Contam. 24(10):1076-87.